Serial Number: 10/664,341 Filing Date: September 16, 2003

Title: RAPIDLY DEGRADED REPORTER FUSION PROTEINS

## **IN THE CLAIMS**

Please amend the claims as follows:

- 1. (Currently Amended) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a fusion polypeptide comprising a reporter protein and at least two different emplementing heterologous protein destabilization sequences both of which are C-terminal to the reporter protein, wherein one heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, or SEQ ID NO:98, wherein another heterologous protein destabilization sequence includes a sequence enriched in proline, glutamic acid, serine, and/or threonine residues (a PEST sequence), wherein the reporter protein is luciferase, fluorescent protein, chloramphenicol acetyltransferase, beta-glucuronidase or beta-galactosidase, and wherein the presence of the at least two different protein destabilization sequences in the fusion polypeptide results in enhanced protein degradation relative to a fusion polypeptide with one of the protein destabilization sequences.
- 2. (Currently Amended) An isolated nucleic acid molecule comprising a nucleic acid sequence comprising an open reading frame for a reporter protein and at least two heterologous destabilization sequences both of which are C-terminal to the reporter protein, wherein one of the heterologous destabilization sequences is a mRNA destabilization sequence and another is a heterologous protein destabilization sequence, wherein one heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, or SEQ ID NO:98, wherein the reporter protein is luciferase, fluorescent protein, chloramphenicol acetyltransferase, betaglucuronidase or beta-galactosidase, and wherein the mRNA destabilization sequence is an AUrich sequence or forms a stem-loop.
- 3. (Currently Amended) An isolated nucleic acid molecule comprising a nucleic acid sequence comprising an open reading frame for a luciferase and at least one heterologous destabilization sequence, wherein a majority of codons in the open reading frame for the

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luciferase are codons which are preferentially employed in a mammalian host cell, wherein if one of the heterologous destabilization sequences is a mRNA destabilization sequence, the mRNA destabilization sequence is an AU-rich sequence or forms a stem-loop, and wherein if one of the heterologous destabilization sequences is a protein destabilization sequence, the heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, or a sequence enriched in proline, glutamic acid, serine, and/or threonine residues.

- 4. (Original) The isolated nucleic acid molecule of claim 1, 2 or 3 further comprising a promoter operably linked to the nucleic acid sequence.
- (Original) The isolated nucleic acid molecule of claim 4 wherein the promoter is a 5. regulatable promoter.
- 6. (Original) The isolated nucleic acid molecule of claim 5 wherein the promoter is an inducible promoter.
- 7. (Original) The isolated nucleic acid molecule of claim 5 wherein the promoter is a repressible promoter.
- 8. (Original) The isolated nucleic acid molecule of claim 1 further comprising a heterologous mRNA destabilization sequence.
- 9. (Original) The isolated nucleic acid molecule of claim 2 or 8 wherein the mRNA destabilization is 3' to the nucleic acid sequence.
- 10. (Previously Presented) The isolated nucleic acid molecule of claim 1 or 2 wherein the nucleic acid sequence encoding at least the reporter protein is optimized for expression in a eukaryotic cell.

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- (Original) The isolated nucleic acid molecule of claim 1 or 2 wherein the reporter protein 11. encodes a luciferase.
- (Withdrawn) The isolated nucleic acid molecule of claim 1 wherein the reporter protein 12. encodes a beetle luciferase.
- (Withdrawn) The isolated nucleic acid molecule of claim 12 wherein the reporter protein 13. encodes a click beetle luciferase.
- 14. (Withdrawn) The isolated nucleic acid molecule of claim 1 wherein the reporter protein encodes an anthozoan luciferase protein.
- 15. (Original) The isolated nucleic acid molecule of claim 3 wherein the heterologous destabilization sequence is a protein destabilization sequence.
- 16. (Original) The isolated nucleic acid molecule of claim 3 wherein the heterologous destabilization sequence is a mRNA destabilization sequence.
- 17. (Original) The isolated nucleic acid molecule of claim 1, 2 or 3 wherein nucleic acid sequence comprises SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, or a fragment thereof that encodes a fusion polypeptide with substantially the same activity as the corresponding full-length fusion polypeptide encoded by SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.
- (Original) The isolated nucleic acid molecule of claim 1 further comprising a mRNA 18. destabilization sequence.

- 19. (Original) The isolated molecule of claim 18 wherein one protein destabilization sequence is a PEST sequence.
- (Previously Presented) The isolated nucleic acid molecule of claim 2 further comprising 20. a heterologous protein destabilization sequence that includes a sequence enriched in proline, glutamic acid, serine, and/or threonine residues (a PEST sequence).
- 21. (Withdrawn) The isolated nucleic acid molecule of claim 1 or 2 wherein one heterologous protein destabilization sequence is from the C-terminus of a mammalian ornithine decarboxylase.
- 22. (Withdrawn) The isolated nucleic acid molecule of claim 1 or 2 wherein one heterologous protein destabilization sequence is a mutant ornithine decarboxylase sequence.
- 23. (Withdrawn) The isolated nucleic acid molecule of claim 21 wherein the mutant ornithine decarboxylase sequence has an amino acid substitution at a position corresponding to position 426, 427, 428, 430, 431, 433, 434, 439 or 448 of murine ornithine decarboxylase.
- 24. (Canceled)
- 25. (Original) The isolated nucleic acid molecule of claim 1 or 2 wherein one heterologous protein destabilization sequence is at the C-terminus of the reporter protein.
- 26. (Withdrawn) The isolated nucleic acid molecule of claim 1 or 2 wherein one heterologous protein destabilization sequence at the N-terminus of the reporter protein.
- 27. (Withdrawn) The isolated nucleic acid molecule of claim 1 or 2 further comprising an ubiquitin polypeptide at the N-terminus of the fusion polypeptide.
- 28. (Withdrawn) The isolated nucleic acid molecule of claim 27 wherein one of the heterologous protein destabilization sequences is at the C-terminus of ubiquitin.

- (Withdrawn) The isolated nucleic acid molecule of claim 28 wherein one of the 29. heterologous protein destabilization sequences comprises a glutamic acid or arginine residue.
- 30. (Original) The isolated nucleic acid molecule of claim 10 which encodes a fusion polypeptide with a half-life of expression of about 20 minutes.
- (Original) The isolated nucleic acid molecule of claim 10 which encodes a fusion 31. polypeptide with a half-life of expression of about 30 minutes.
- 32. (Original) The isolated nucleic acid molecule of claim 15 wherein the heterologous protein destabilization sequence is a PEST sequence.
- 33. (Withdrawn) The isolated nucleic acid molecule of claim 15 wherein the heterologous protein destabilization sequence is from the C-terminus of a mammalian ornithine decarboxylase.
- 34. (Previously Presented) The isolated nucleic acid molecule of claim 15 wherein the heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 or SEQ ID NO:98.
- 35. (Original) A vector comprising the nucleic acid molecule of claim 1, 2 or 3.
- 36. (Original) The vector of claim 35 wherein the nucleic acid molecule is operably linked to a regulatable promoter.
- 37. (Original) The vector of claim 36 wherein the promoter is a repressible promoter.
- 38. (Withdrawn) The vector of claim 34 wherein the nucleic acid molecule comprises SEO ID NO:49, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80 or a fragment thereof that encodes a fusion polypeptide with substantially the

same activity as the corresponding full-length fusion polypeptide encoded by SEQ ID NO:49, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.

- (Withdrawn) A fusion polypeptide encoded by the nucleic acid molecule of claim 1, 2 or 39. 3.
- 40. (Withdrawn) The fusion polypeptide of claim 38 wherein the reporter protein is chloramphenicol acetyltransferase, luciferase, beta-glucuronidase or beta-galactosidase.
- 41. (Original) A host cell comprising the vector of claim 35.
- 42. (Original) The host cell of claim 41 which is stably transfected with the vector that encodes a fusion polypeptide comprising a luminescent protein.
- 43. (Original) The host cell of claim 42 wherein the signal emitted by the host cell comprising the vector is greater than the signal emitted by a corresponding host cell comprising a vector which lacks one or more of the destabilization sequences.
- 44. (Original) A stable cell line comprising the vector of claim 35 wherein the signal emitted by the reporter protein is equal to or greater than a signal emitted by a corresponding stable cell line comprising a vector which lacks one or more of the heterologous destabilization sequences.
- 45. (Withdrawn) A method to detect a reporter protein in a cell, comprising:
  - a) contacting a cell with the vector of claim 35; and
- b) detecting or determining the presence or amount of the reporter protein in the cell or a lysate thereof.
- 46. (Withdrawn) The isolated nucleic acid molecule of claim 1 or 2 wherein the reporter protein is green fluorescent protein, chloramphenicol acetyltransferase, beta-glucuronidase or beta-galactosidase.

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AMENDMENT AND RESPONSE UNDER 37 C.F.R. § 1.111

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47. (Canceled).